

# SOME OBSERVATIONS ON LEAF FORM IN *ILEX VOMITORIA* (AQUIFOLIACEAE)

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## ABSTRACT

We report a statistical analysis of leaf form for a sample of 481 collected from five yaupon trees at Ponchatoula, Louisiana. Considerable variation exists within these "sun" leaves. Across the 5 trees, surface area varies 24-fold (22.1–533 mm<sup>2</sup>), leaf specific mass 22-fold (7.67–167.83 g m<sup>-2</sup>), leaf length 5-fold (6–31 mm), leaf width 4.5-fold (4–18 mm), and crenations 4.7-fold (7–33 per leaf). Leaf complexity (LC) varies from 1 to 6 Fourier frequencies, and the leaf dissection index (DI) varies from a nearly circular 1.036 to a high of 1.349. Trees in this population are statistically significantly different from each other in average leaf size, leaf mass, leaf specific mass, and in number of crenations per leaf. In logistic regression, the probability that a leaf will develop more crenations, increases with leaf size. As leaf size changes, shape remains relatively constant within this population; amount of leaf dissection does not correlate with other morphological variables. Leaf surface area is strongly related to nodal position on the shoot, middle nodes generally produce the largest leaves.

KEY WORDS: *Ilex vomitoria*, yaupon, leaf form

## RESUMEN

Se realiza un análisis estadístico de la forma de la hoja sobre una muestra de 481 hojas colectadas en cinco árboles en Ponchatoula, Louisiana. Existe una variación considerable entre esta hojas de "sol." En cinco árboles, el área de la superficie varía en 24 veces (de 22.1–533 mm<sup>2</sup>), la masa específica de la hoja en 22 veces (de 7.67–167.83 g m<sup>-2</sup>), la longitud foliar en cinco veces (de 6–31 mm), la anchura de la hoja en 4.5 veces (de 4–18 mm), y las crenaciones en 4.7 veces (de 7–33 por hoja). La complejidad de la hoja (LC) varía de 1 a 6 frecuencias de Fourier, y el índice de disección foliar (DI) varía desde casi circular 1.036 hasta 1.349. Los árboles de esta población muestran diferencias significativas estadísticamente en el tamaño foliar medio, masa foliar, la masa foliar específica, y en el número de crenaciones por hoja. En regresión logística, la probabilidad de que una hoja desarrolle más crenaciones aumenta con el tamaño de la hoja. Cuando varía el tamaño de la hoja, su forma permanece relativamente constante en esta población; la disección de la hoja no se correlaciona con otras variable morfológicas. El área de la superficie foliar está fuertemente relacionada con la posición nodal en la rama, los nudos medios producen generalmente las hojas más grandes.

## INTRODUCTION

Advances in computer-assisted image analysis expand the ability of botanists to use large sample sizes in leaf morphometric research (e.g., Kincaid and Schneider 1983, White et al. 1988). Our objective was to quantify leaf variability in a population of yaupon, *Ilex vomitoria* Aiton (Aquifoliaceae) at Ponchatoula, Louisiana. We collected leaves from five trees in order to answer these questions: (1) Are trees homogeneous in leaf size and shape? (2) Is leaf form related to nodal position along the twig? (3) Do predictive relationships exist among leaf specific mass (g dry weight / m<sup>2</sup> surface area), crenation number, mass, area, dissection index, and leaf shape complexity? (4) How do average leaf images per tree, reconstructed by Fourier transform, compare to conventional morphometric statistical analysis?

Yaupon is a shrub and small tree common in forests along the Coastal Plain from southern Virginia to Florida, and west to Texas (Elias 1980). The leaves are small, flat, coriaceous, evergreen, elliptical, and have marginal mucronate crenations (Radford et al. 1968).

## METHODS

A sample of 481 leaves was collected, on August 12, 1989, from 5 trees growing within 100 meters of each other along a sunlit edge of a pine forest at Ponchatoula, Louisiana. Leaves were individually numbered with a serial number and nodal position on the current year's shoot, the petiole excised, and the blades placed into a plant press. After drying in a convection oven at 70°C, the leaves were weighed to the nearest 0.0001 gram. Crenations were counted using a stereo dissecting microscope. Maximum length and width were recorded for each blade.

In our laboratory, leaf images are analyzed (Fig. 1) using the leaf boundary method of Kincaid and Schneider (1983) which is based on Fourier transform. In an analysis of various computerized leaf morphometric methods, White, et al. (1988) found this method performed well in terms of discriminating power and in the reconstruction of synthetic, average leaf images.

Leaves were photographically enlarged (Fig. 2), and the images boundaries digitized into x, y coordinates using a graphics pad (Model CR1212, Summagraphics Corp., Fairfield, CT) attached to a Macintosh IICI computer (Fig. 1). Other details are in Kincaid and Schneider (1983), and in Figures 1–3. Image information lies in the values of the Fourier coefficients at each frequency (Table 1). Leaves have the same size and shape, if and only if, their Fourier coefficients are identical (Kincaid and Schneider 1983). Using this method, leaf surface area, leaf complexity, and leaf dissection index were computed for 342 out of the 481 leaves. Leaf complexity (LC) is a dimensionless and discrete, ordinal variable providing a mathematical measure of the “complexity” of a leaf's outline. For example, LC = 1 for pure ellipses, and

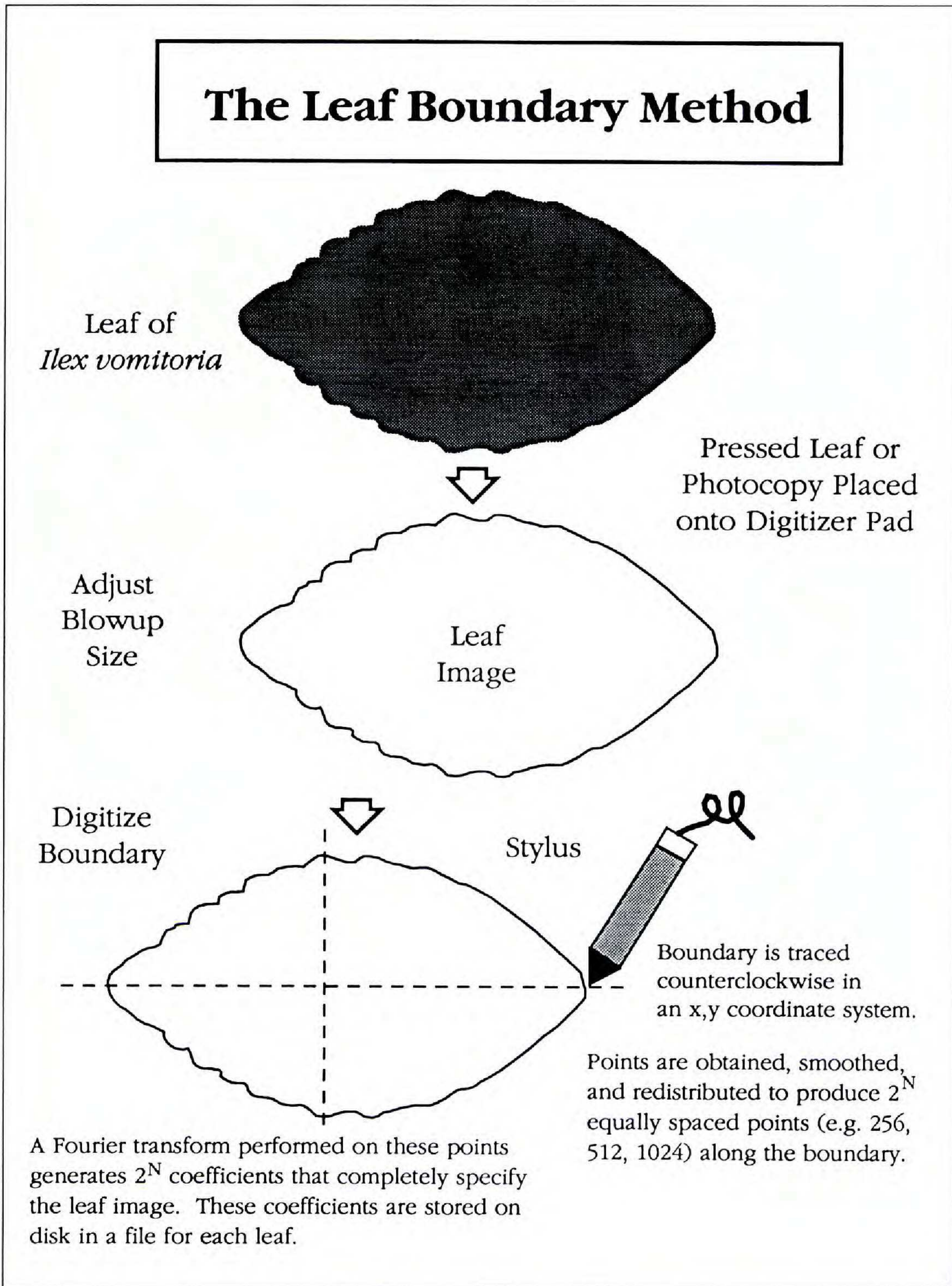


FIG. 1. Leaf boundary method of Kincaid and Schneider (1983).

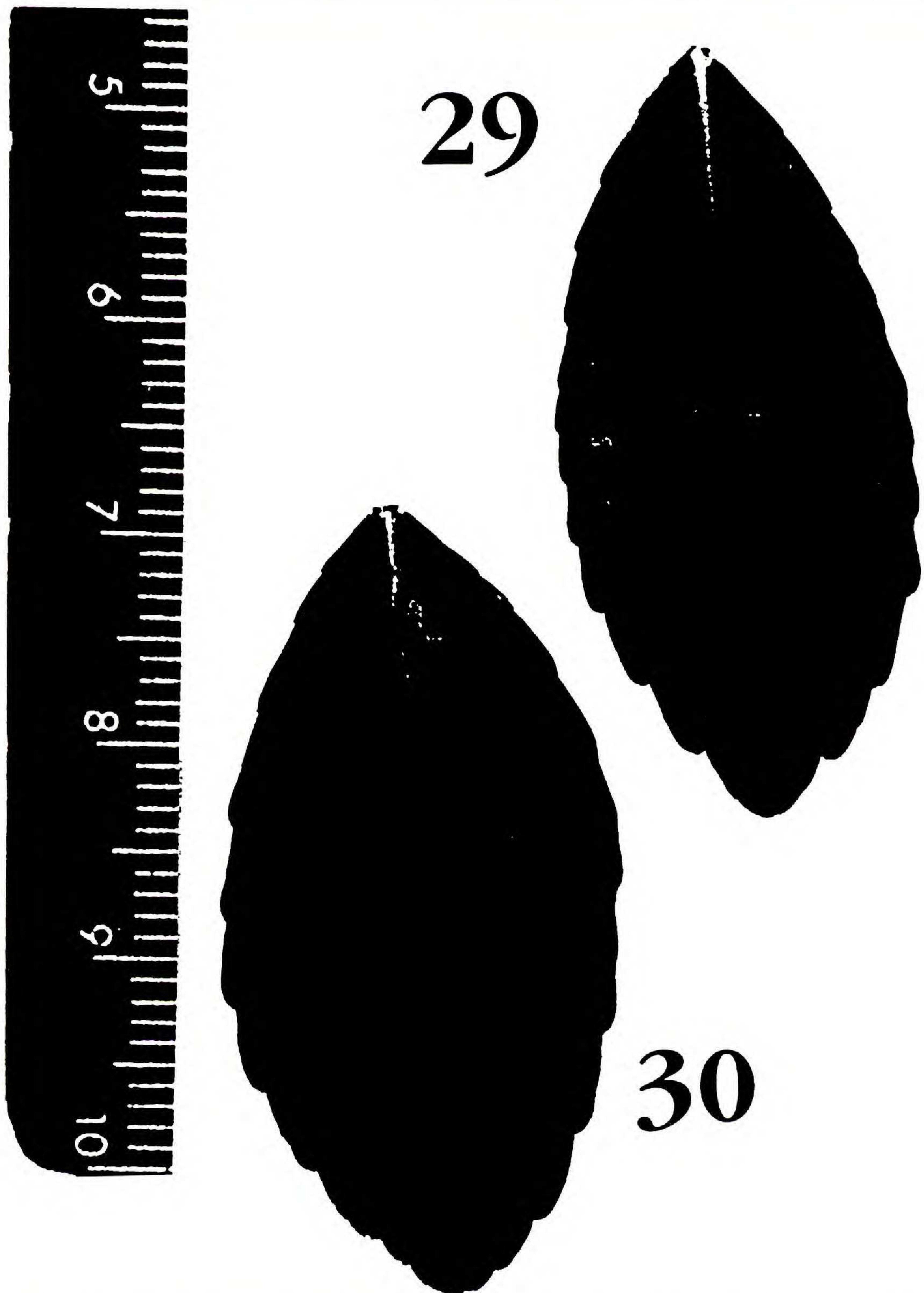


FIG. 2. Enlarged, photographic image of leaf #30 with ruler (small divisions are mm), as digitized. (Flat-bed scanners have now replaced photography as a preferred method of image capture and manipulation prior to digitization of boundary and Fourier Transform.)

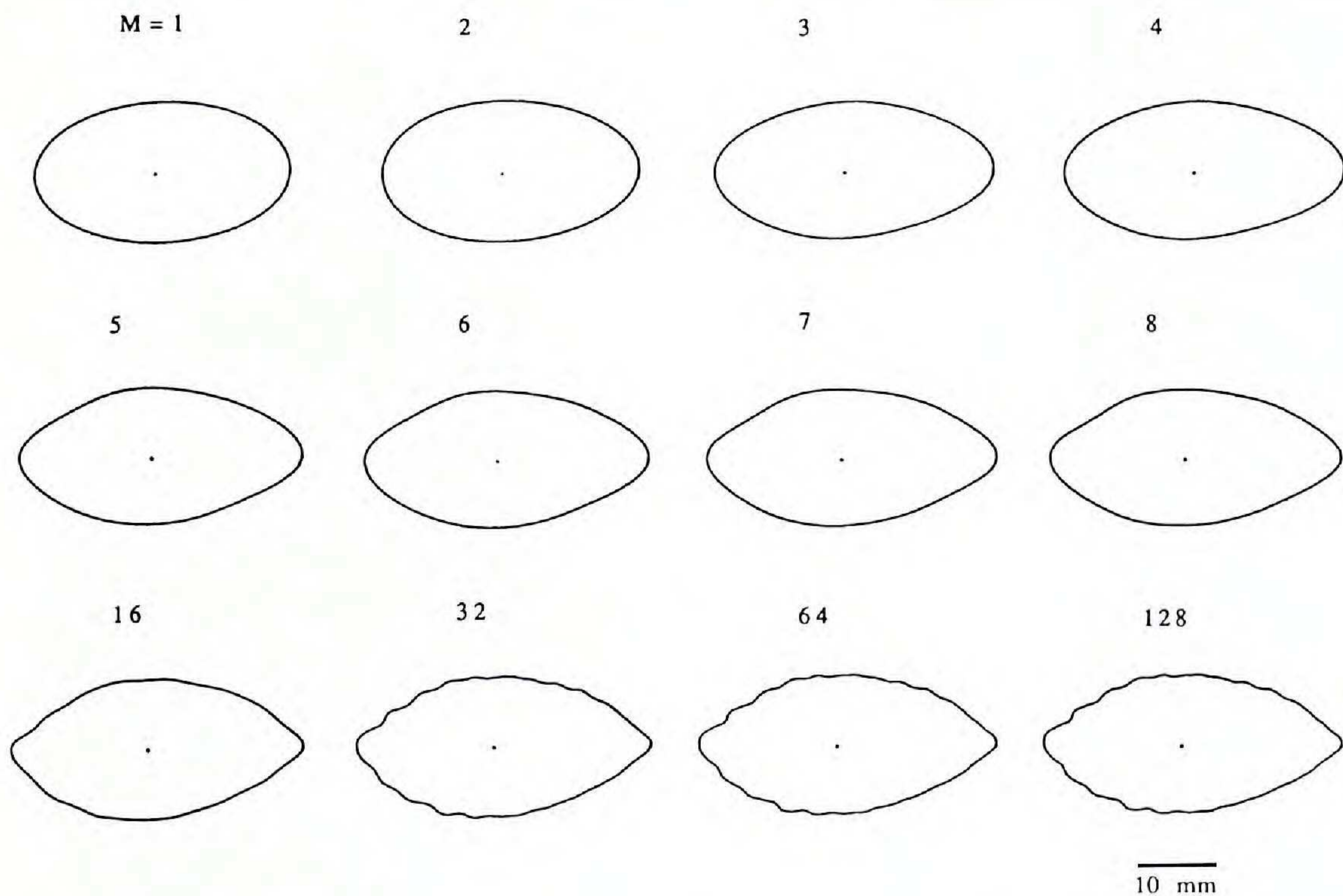


FIG. 3. Images of leaf #30 are reconstructed by reverse Fourier Transform, using various frequencies ( $M$ ). Reconstruction with  $M = 1$  (the first frequency) yields the “best fit” ellipse.  $M = 3$  reconstructs leaf #30 to within 95% of a full reconstruction ( $M = 128$ ). The finer details of the leaf margin are encoded by the higher frequencies. Notice the image transition between  $M = 16$  and  $M = 32$ , and that frequencies greater than  $M = 32$  add no detectable marginal detail. For leaves of simple shape, and for studies such as this one where detailed image analysis of mucronate crenations is not paramount, one need only deal with coefficients from the lower frequencies.

$LC = 1$  for most elliptically shaped leaves with relatively smooth margins. The more complicated the leaf shape, relative to the best fit ellipse, the greater the integer value of  $LC$  (In terms of reverse Fourier transform,  $LC$  is the number of frequencies necessary to reconstruct the leaf image to within 95% of the actual image.).

While  $LC$  captures a mathematical aspect of leaf shape, dissection index ( $DI$ ) is the empirical relationship between leaf perimeter and leaf surface area expressed as a dimensionless, continuous measurement variable, perhaps amenable to biophysical interpretation of convective heat exchange. The minimum value of  $DI$  is that of a circle for which  $DI$  always equals 1.0. The value of  $DI$  for a circularly shaped, entire leaf is slightly larger than 1.0, and the more deeply lobed, dissected or lanceolate a leaf shape, the larger the value [ $DI = \text{Perimeter} / \{2 \cdot \text{SRQ}(\text{Area} \cdot \pi)\}$ ]. These variables, defined by Kincaid and Schneider (1983), are useful in making comparisons of leaf shape among leaves having different surface areas.

TABLE 1. Fourier coefficients in millimeters for an individual leaf (# 30) of yaupon displayed for the first 14 frequencies (out of a total of  $M = 128$  frequencies).  $M = 3$  encodes 95% of this image and  $M = 8$  is sufficient for data analysis for this species. Reconstruction of the image of a leaf using  $M = 1$  (the +1 and -1 freq.) yields the best fit ellipse while reconstructions using more frequencies yield finer and finer marginal details. If the Fourier coefficients are for a circle, the +1 real coefficient is the radius and all other coefficients are zero.

Fourier Frequency	Real part	Coefficients	Imaginary part
1	12.34		-.26
-1	3.53		.26
2	.02		0
-2	.19		.02
3	.75		-.11
-3	.48		.01
4	.06		.02
-4	0		-.01
5	.25		-.09
-5	.20		-.02
6	0		.03
-6	.02		.01
7	.12		-.03
-7	.09		0
8	-.01		.01
-8	.02		.02
9	.08		-.01
-9	.07		.01
10	.01		0
-10	-.02		0
11	.06		-.02
-11	.05		.01
12	-.02		0
-12	.01		0
13	.06		-.02
-13	.03		.01
14	0		0
-14	0		.01

We used StatView (Abacus) and JMP (SAS 1989) on Macintosh computers to perform the data analysis. The technique of logistic regression (Pagano and Gauvreau 1993; using JMP) was utilized to search for trends, and to visualize relationships between crenation interval (dependent variable) and leaf surface area, and between classification of leaves by tree (dependent variable) and number of leaf crenations. While logistic regression is a commonly used statistical tool in biomedical fields for analyzing discrete responses, it is rarely used in organismic biology.

#### RESULTS AND DISCUSSION

Based on our sample of 481 leaves, considerable leaf variation exists within these "sun" leaves. Across the 5 trees, surface area varies 24-fold (22.1–533

mm<sup>2</sup>), leaf specific mass 22-fold (7.67–167.83 g m<sup>-2</sup>), leaf length 5-fold (6–31 mm), leaf width 4.5-fold (4–18 mm), crenations 4.7-fold (7–33 per leaf), and leaf complexity (LC) from 1 to 6 Fourier frequencies. Leaf dissection index (DI) changes from a nearly circular 1.036 to a high of 1.349. Table 2 provides descriptive statistics and results of analysis of variance for these leaves grouped by tree.

We found strong evidence for heterogeneity among the five trees for average leaf area, mass, leaf specific mass, crenation number, and dissection index ( $P < 0.00001$  with R-square values for the main effect ranging from 0.153–0.542, Table 2). Interestingly, average LC was homogeneous among the 5 trees ( $F = 1.7$ ; 4,338 df;  $P = 0.14$ ) and homogeneous among shoots within each tree ( $P > 0.05$ ). We predicted that leaf dissection, a variable providing linkage to convective cooling ability, would be positively correlated with leaf surface area. However leaf dissection did not correlate with any variable, indicating that as leaf size changes, shape remains relatively constant within this population.

In these “sun” leaves, leaf weight ( $r = 0.91$ ), leaf specific mass ( $r = 0.24$ ), and crenation number ( $r = 0.48$ ) increased with leaf size ( $P < 0.01$  for each correlation coefficient). Figure 4 presents the relationship between leaf mass and leaf area. On an individual tree basis, dry weight of leaf is an excellent predictor of surface area (e.g., in Figure 5 for Tree 5, R-square = 0.976) but less so for all leaves (R-square = 0.826). As a field technique, leaf width provides the simplest predictor of leaf surface area (e.g., for Trees 1 & 4,  $\text{Area} = 25.30 * \text{Width} - 60.46$ , R-sq. = 0.91). Once it is determined that leaf shape changes little with leaf area, regression equations could be used to predict leaf area, as dry weight and/or blade width is easier to measure than leaf area.

Increased leaf specific mass usually confers greater water use efficiency and photosynthetic capacity. Average leaf specific mass ranged from 82.6 in tree 4, to 141.3 g m<sup>-2</sup> in tree 5. We cannot explain why leaf specific mass varied so much in this study, among 5 trees growing within 100 meters of each other (Table 1). Indeed, trees 4 and 5, with essentially the same average leaf images (Fig. 8), had the most divergent values for leaf specific mass.

Crenation number, grouped into 5 levels (7–10, 11–14, 15–18, 19–22, and 23–33 crenations per blade), was declared a “response” variable, and analyzed by logistic regression against leaf surface area as an explanatory variable (Figure 5). In logistic regression, crenation value is not predicted, rather, probabilities are estimated for each level of crenation “response,” given leaf surface area. The resultant graph, partitions the outcome space into mutually exclusive regions. To use the logistic regression graph, one draws a vertical line at any desired surface area dividing the estimated probability into segments for each level of response. For example, as leaf size increases beyond 200 mm<sup>2</sup>, the probability that a leaf will have 7–10

TABLE 2. Descriptive statistics (mean and SD) with single classification analysis of variance for leaf morphometric variables for five trees of yaupon collected August 12, 1989, Ponchatoula, LA. Surface area is mm<sup>2</sup> for one side; mass is mg dry weight; leaf specific mass is g dry weight / m<sup>2</sup> surface area; dissection index is a dimensionless number for perimeter relative to surface area with circles having a DI = 1 (Dissection Index = Perimeter / (2 \* SQR(Area \* pi))); leaf complexity is the number of Fourier frequencies necessary to reconstruct a leaf image to within 95% of a complete reconstruction. R-square is the proportion of the variability (total sum of squares) in each variable accounted for by the main effect (leaves grouped by tree).

VARIABLE	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	F	ONE-WAY ANOVA		R-square
							df	P	
Surface Area	138.2 (53.09)	254.6 (115.50)	130.1 (39.34)	98.5 (33.17)	92.6 (22.33)	71.8	4, 337	<.00001	0.460
Leaf Mass	9.3 (.006)	31.6 (.017)	12.1 (.004)	6.9 (.003)	13.4 (.004)	140.9	4, 476	<.00001	0.542
Leaf Specific Mass	92.8 (27.76)	117.7 (19.74)	92.4 (11.84)	82.6 (29.56)	141.3 (15.74)	55.3	4, 338	<.00001	0.396
Crenation Number	14.5 (2.52)	17.7 (4.49)	13.3 (2.52)	16.2 (2.99)	15.7 (5.48)	21.5	4, 476	<.00001	0.153
Dissection Index	1.13 (.039)	1.14 (.500)	1.24 (.060)	1.16 (.041)	1.15 (.022)	63.5	4, 338	<.00001	0.429
Leaf Complexity	2.95 (.580)	2.97 (.701)	3.16 (.541)	3.07 (.464)	3.09 (.384)	1.7	4, 338	0.14 ns	0.020



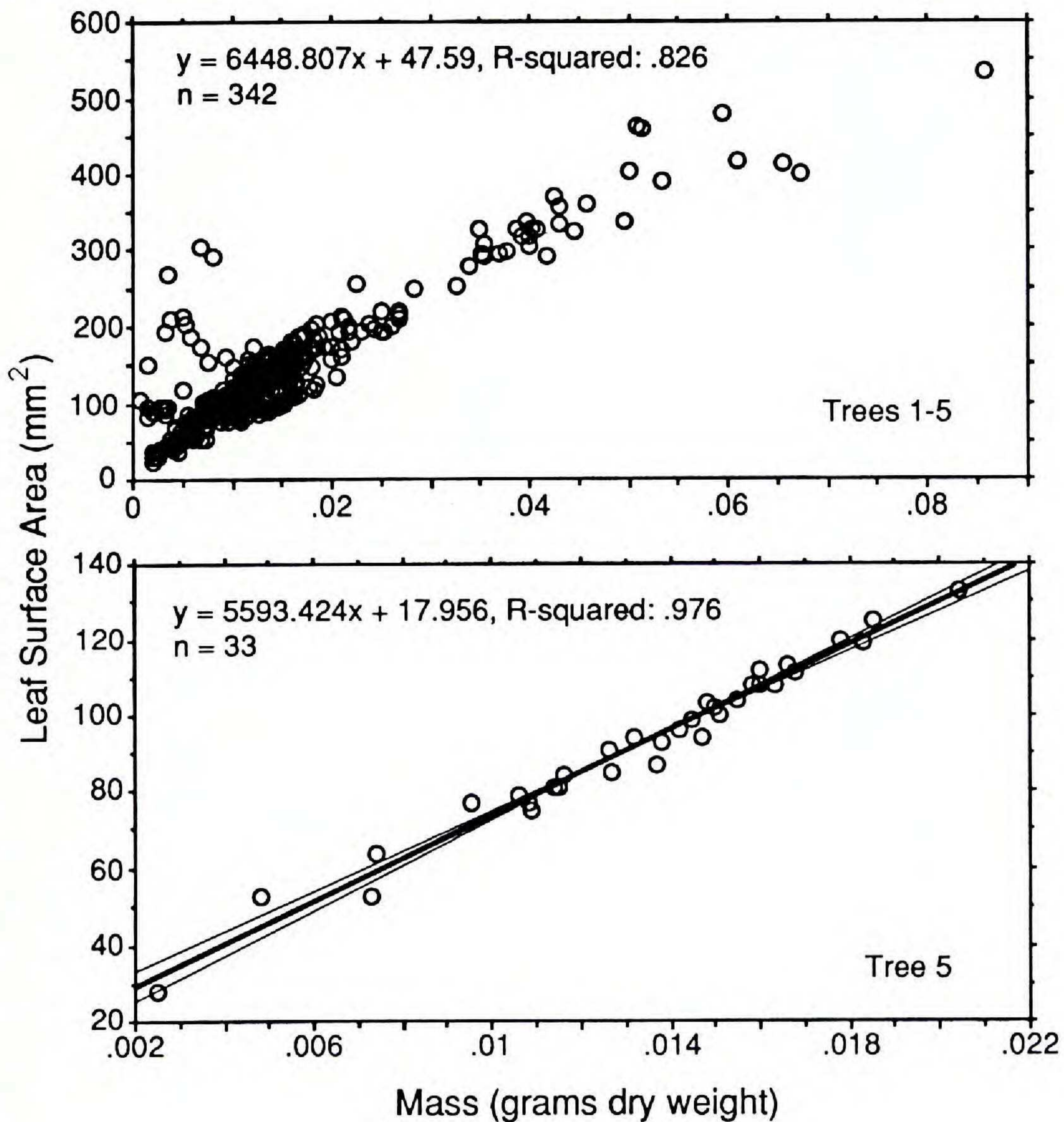


FIG. 4. Relationship between leaf surface area and leaf mass. Upper graph: 5 trees ( $n = 342$ ); outliers (less than 0.02 g) are immature leaves. Lower graph: Tree 5 ( $n = 33$ ). Least squares linear regression linear regression ( $P < 0.0001$  for both) with 95% confidence intervals for slope given for Tree 5.

crenations becomes extremely low. Large leaves ( $> 400 \text{ mm}^2$ ) are much more likely to develop 15–33 crenations than they are likely to develop 7–18 crenations.

In Figure 6, we use logistic regression as a tool to visualize the estimated probability of tree “membership” for a leaf, given the number of crenations on a particular leaf. Trees 1 and 3 have the lowest average number of crenations per leaf; and Trees 2 and 4 have the largest average number (Table 2). The fitted logistic regression curves of Figure 6 provide a display of these trends across the entire range of number of crenations found in this study.

Leaf surface area is strongly related to nodal position, but only when

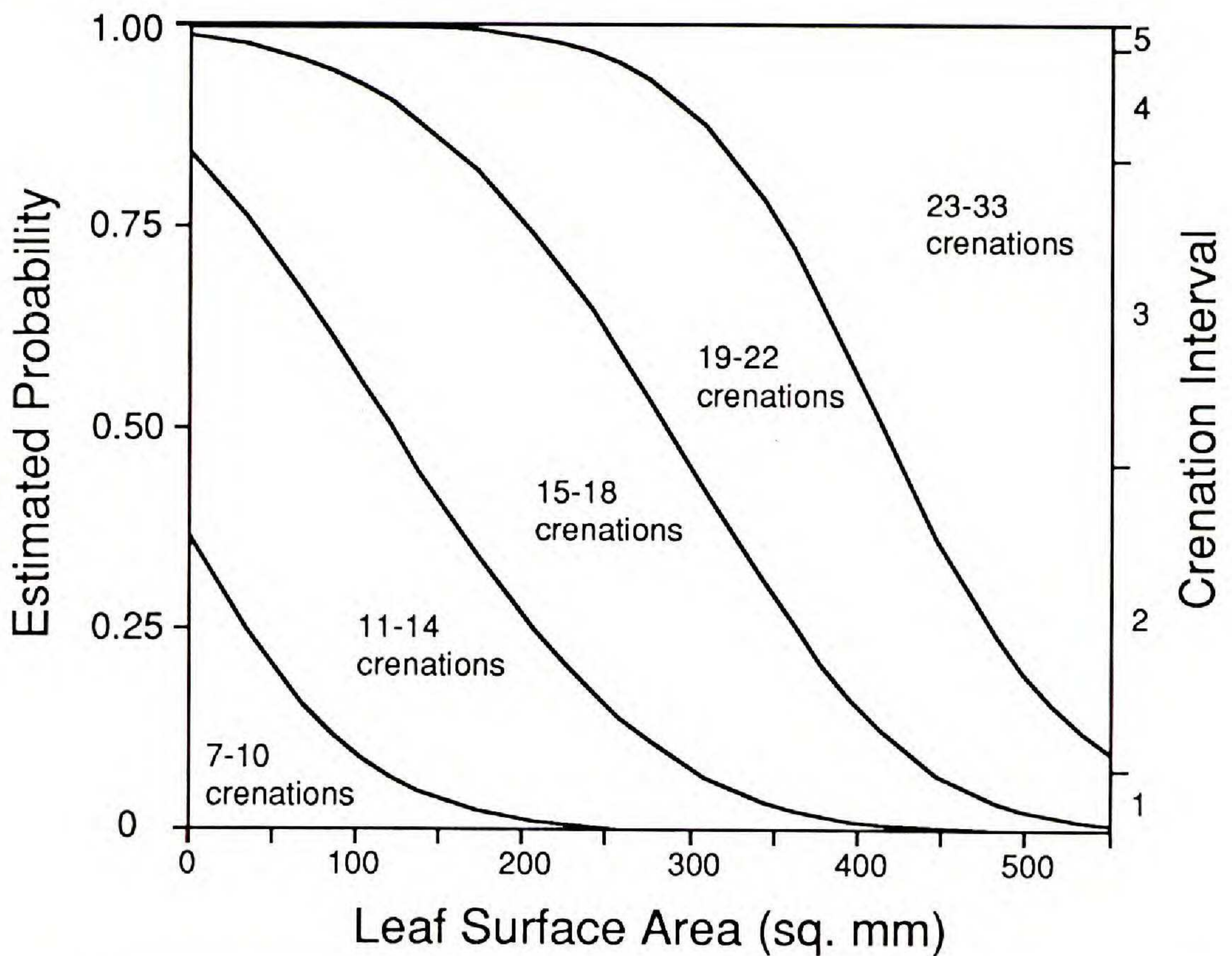


FIG. 5. Logistic regression analysis of number of crenations versus leaf surface area as the explanatory variable. In analysis of loglikelihood, chi-square = 117.4,  $P < 0.00001$ ,  $N = 339$ .

analyzed on a per shoot basis (Fig. 7). For many shoots, 70–90% (R-square) of total variability in leaf area is accounted for by nodal position. This holds true even for long terminal shoots that have experienced, over the growing season, an episodic growth and/or a developmental switch from preformed to neo-formed (produced and released in current growing season) leaf buds (lower right graph in Fig. 7). Shoots within trees had the same average leaf size in ANOVA.

Average leaf images reconstructed for each tree, as computed from average Fourier coefficients for the first 8 frequencies, are displayed in Figure 8 along with principal component analysis of more conventional morphological variables. The Fourier transform captures only the two-dimensional leaf outline: We see from these average images that Trees 4 and 5 have very similar leaf sizes and shapes, and that Trees 1 and 3 have similar leaf sizes but different shapes. In principal component analysis, leaf area loaded heavily on the first component which accounted for 0.33 of total variance. The shape variables (DI and LC) loaded on the second component, accounting for 0.28 of the variance. Tree number, loaded on the third component, accounting for 0.19 of the variance. As a general rule for biological objects analyzed by

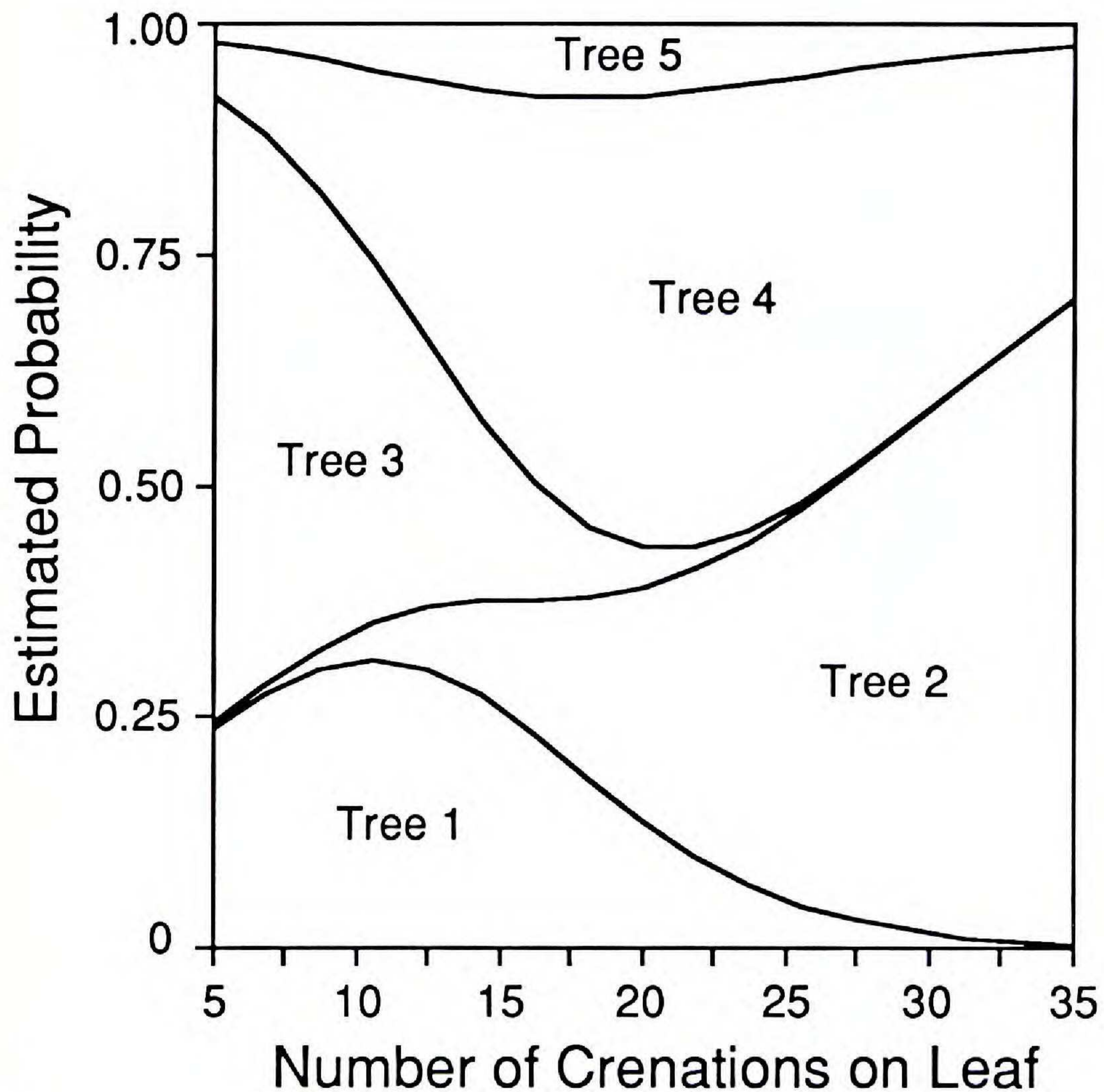


FIG. 6. Visualization of relationship between probability of tree “membership” and number of leaf crenations by logistic regression analysis (analysis of loglikelihood chi-square = 84.2,  $P < 0.00001$ ,  $N = 481$ ).

principal components, “size” variables dominate the first component and “shape” variables the second component (Pimentel 1979). The scatterplot of the 342 leaves, graphed in PC space for the first two components, revealed clusters of points corresponding to tree. The 95% confidence ellipses of the bivariate means of each of these clusters are displayed in the lower part of Figure 8. Trees 3, 4 and 5 cluster rather closely together, while Trees 1 and 2 are distinct.

Leaf surface area appears to be functionally related to nodal position, with the middle nodes producing leaves that often are 15 to 20 times larger in surface area than leaves at the early and late nodes (Fig. 7). This range in leaf surface area within single shoots, may transcend average differences from populations across the latitudinal and sun-shade extremes of the species.

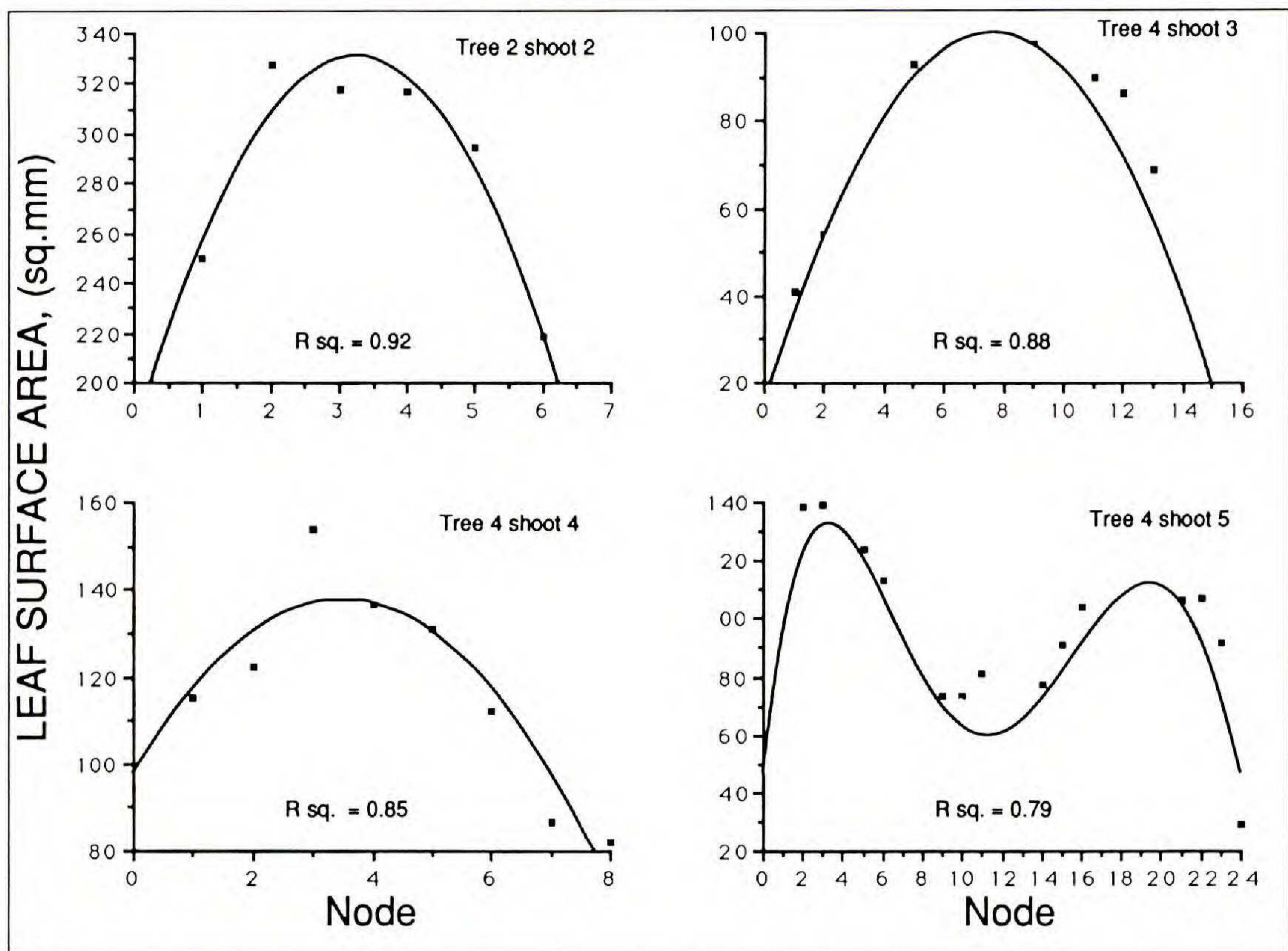


FIG. 7. Leaf surface area is functionally related to nodal position. Seventy to ninety percent (R-square in second order polynomial regression) of the variability in area is accounted for by nodal position. This remains true even for long terminal shoots that have experienced, over the growing season, episodic growth and/or a developmental switch from preformed to neo-formed leaf buds (lower right figure).

We have established nine study populations of yaupon. Three of these are inland stations: Aiken, South Carolina; Homosassa Springs, Florida; and, the subject of this paper, Ponchatula, Louisiana. We have also established six coastal stations from the species northern distributional limit, Virginia Beach, Virginia, to one of its southern limits, New Smyrna Beach, Florida. Exploratory analysis of the modestly sized data set of this paper has helped us plan collection strategies for the other stations. As botanical methods of computer-assisted image analysis advance (White et al. 1988), research designs should be able to accommodate larger sample sizes of leaves, involving more trees per population, and more shoots per tree.

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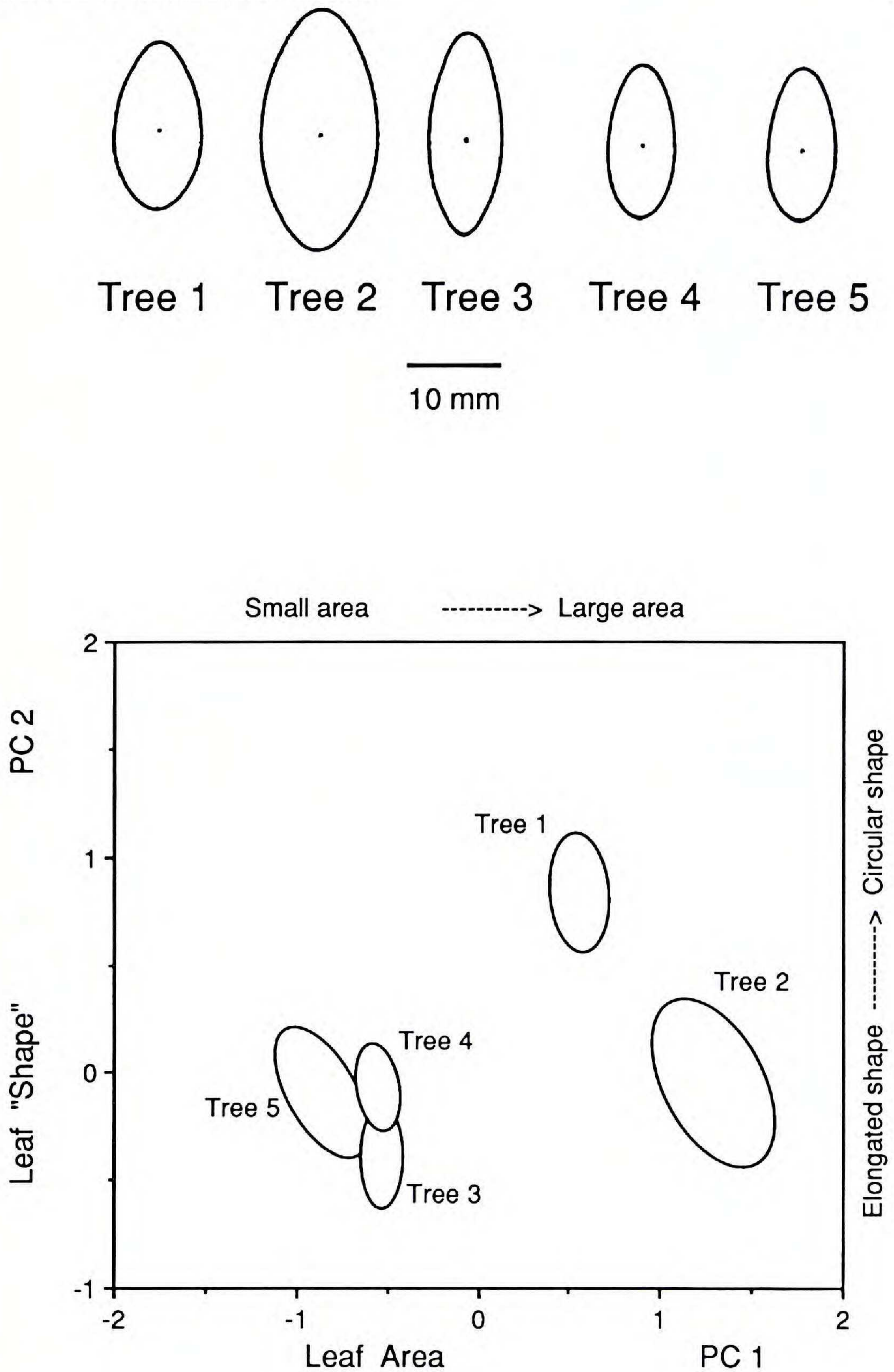


FIG. 8. Upper: Average leaf images per tree reconstructed from average Fourier coefficients. Lower: Principal component analysis with 95% confidence ellipses of the bivariate means for each Tree, N = 342 leaves.

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